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Report Title

Enzymatic decontamination of environmental organophosphorus compounds

ABSTRACT

This final report describes the research performed during the award period (June 5, 2003 to December 4, 2006).
The main thrust of the research project was to investigate the surface chemistry of and molecular interactions between OP compounds and enzymes. The objective were:
i) to characterize the Langmuir films of organophosphorus acid hydrolase (OPH) and organophosphorus acid anhydrase (OPAA) by studying their interfacial and spectroscopic properties at air/water interface;
ii) to investigate the molecular interactions between OPH and OPAA and the OP compounds using Polarization Modulation FTIR spectroscopy;
iii) to characterize the topography of OPH and OPAA by scanning probe and environmental scanning electron microscopies;
iv) to characterize the basic features of AChE biosensor based on LB film technology.
v) to simulate the active site of acetylcholinesterase (AChE)

List of papers submitted or published that acknowledge ARO support during this reporting period. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

1. Constantine, C. A.; Mello, S. V.; Dupont, A.; Cao, X.; Santos, D., Jr.; Oliveira, O. N., Jr.; Strixino, F. T.; Pereira, E. C.; Cheng, T.-C.; DeFrank, J. J.; Leblanc, R. M. “Layer-by-Layer Self-Assembled Chitosan/Poly(thiophene-3-acetic acid) and Organophosphorus Hydrolase Multilayers” J. Am. Chem. Soc., 2003, 125(7), 1805-1809.

2. Constantine, C. A.; Gattas-Asfura, K. M.; Mello, S. V.; Crespo, G.; Rastogi, V.; Cheng, T.-C.; DeFrank, J. J.; Leblanc, R. M. “Layer-by-Layer Films of Chitosan, Organophosphorus Hydrolase and Thioglycolic Acid-Capped CdSe Quantum Dots for the Detection of Paraoxon” J. Phys. Chem. B., 2003, 107(50), 13762-13764.

3. Constantine, C. A.; Gattas-Asfura, K. M.; Mello, S. V.; Crespo, G.; Rastogi, V.; Cheng, T.-C.; DeFrank, J. J.; Leblanc, R. M. “Layer-by-Layer Biosensor Assembly Incorporating Functionalized Quantum Dots” Langmuir, 2003, 19(23), 9863-9867.

4. Mello, S. V.; Mabrouki, M.; Cao, X.; Leblanc, R. M.; Cheng, T.-C.; DeFrank, J. J. “Langmuir and Langmuir-Blodgett Films of Organophosphorus Acid Anhydrolase” Biomacromolecules, 2003, 4(4), 968-973.

5. Zheng, J.; Constantine, C.A.; Rastogi, V.K.; Cheng, T.-C.; DeFrank, J.J.; Leblanc, R.M. "Secondary Structure of Organophosphorus Hydrolase in Solution and in Langmuir-Blodgett Film Studied by Circular Dichroism Spectroscopy" J. Phys. Chem. B., 2004, 108(44), 17238-17242.

6. Cao, X.; Mello, S.V.; Leblanc, R.M.; Rastogi, V.K.; Cheng, T.-C.; DeFrank, J.J. “Detection of paraoxon by immobilized organophosphorus hydrolase in a Langmuir–Blodgett film" Colloids and Surfaces A: Physicochem. Eng. Asp., 2004, 250(1-3), 349-356.

7. Cao, X.; Mabrouki, M.; Mello, S.V.; Leblanc, R.M.; Rastogi, V.K.; Cheng, T.-C.; DeFrank, J.J. "The interaction between OPH and paraoxon at the air–water interface studied by AFM and epifluorescence microscopies" Colloids and Surfaces B: Biointerfaces, 2005, 40(2), 75-81.

8. Zheng, J.; Constantine, C.A.; Zhao, L.; Rastogi, V.K.; Cheng, T.-C.; DeFrank, J.J.; Leblanc, R.M. "Molecular Interaction between Organophosphorus Acid Anhydrolase and Diisopropylfluorophosphate” Biomacromolecules, 2005, 6(3), 1555-1560.

9. Ji, X.; Zheng, J.; Xu, J.; Rastogi, V.K.; Cheng, T.-C.; DeFrank, J.J.; Leblanc, R.M. "(CdSe)ZnS Quantum Dots and Organophosphorus Hydrolase Bioconjugate as Biosensors for Detection of Paraoxon" J. Phys. Chem. B., 2005, 109(9), 3793-3799.

10. Wang,C.; Li, C.; Ji, X.; Orbulescu, J.; Xu, J.; Leblanc, R.M. "Peptidolipid as Binding Site of Acetylcholinesterase: Molecular Recognition of Paraoxon in Langmuir Films" Langmuir, 2006, 22, 2200-2204.

11. Xu, J.; Ji, X.; Gattás-Asfura, K.M.; Wang, C.; Leblanc, R.M. "Langmuir and Langmuir–Blodgett films of quantum dots" Colloids and Surfaces A: Physicochem. Eng. Asp., 2006, 284-285, 35-42.

12. Leblanc, R.M. "Molecular recognition at Langmuir monolayers" Curr. Opin. Chem. Biol., 2006, 10, 529-536..

13. Orbulescu, J.; Constantine, C.A.; Rastogi, V.K.; Shah, S.S.; DeFrank, J.J.; Leblanc, R.M. "Detection of Organophosphorus Compounds by Chemically Immobilized Organophosphorus Hydrolase" Anal. Chem., 2006, 78, 7016-7021.

14. Orbulescu, J.; Leblanc, R.M. "Surface engineering Quantum Dots at the Air-water interface" In Particulate Systems in Nano and Biotechnologies, Topics in Applied Physics series, 2006, in press.

15. Zheng, J.; Desbat, B.; Rastogi, V.K.; Shah, S.S.; DeFrank, J.J.; Leblanc, R.M. "Organophosphorus Hydrolase (OPH) at the Air-Water Interface: Secondary Structure and Interaction with Paraoxon" Biomacromolecules, 2006, 7, 2806-2810.

16. Zheng, J.; Leblanc, R.M. "FT-IR at the Air-water Interface" In Advanced Chemistry of Monolayers at Interfaces, Trend in Methodology and Technology, Ed. by Imae, T., Elsevier, 2006, in press.

Number of Papers published in peer-reviewed journals: 16.00

(b) Papers published in non-peer-reviewed journals or in conference proceedings (N/A for none)

N/A

Number of Papers published in non peer-reviewed journals: 0.00

(c) Presentations

CONFERENCE PRESENTATIONS

2005

1. Ji, X., J. Zheng, J. Xu, V.K. Rastogi, T.-C. Chen, J.J. Defrank and R.M. Leblanc. "(CdSe)ZnS QDs and organophosphorus hydrolase bioconjugate as biosensors for detection of paraoxon". 229th ACS National Meeting 9San Diego, CA, March 13-17).

2. Zhao, L., C.A. Constantine, X. Ji, T.-C. Chen and R.M. Leblanc. "A circular dichroism study of OPAA in solution and in Langmuir-Blodgett film". 229th ACS National Meeting (San Diego, CA, March 13-17).

3. Zheng, J., C.A. Constantine, V.K. Rastogi, T.-C. Chen, J.J. DeFrank and R.M. Leblanc. "Molecular interaction between organophosphorus acid anhydrolase (OPAA) and diisopropylfluorophosphate (DFP)". 229th ACS National Meeting (San Diego, CA, March 13-17).

2004

4. Leblanc, R.M., X. Ji, J. Zheng, J. Xu, V.K. Rastogi, T.-C. Cheng and J.J. DeFrank "(CdSe)ZnS quantum dots and organophosphorus hydrolase bioconjugate as biosensor for detection of paraoxon". The 56th Southeast Regional Meeting 2004 (SERMACS) (Sheraton Imperial, Research Triangle Park, Durham, November 10-13).

5. Gattas-Asfura, K.M., C.A. Constantine, G. Sui, M. Micic, S. Magonov and R. M. Leblanc. "Novel optical sensors based on the surface engineering of quantum dots". 228th ACS National Meeting (Philadelphia, PA, August 22-26).

6. Zheng, J., X. Cao, R.M. Leblanc, V.P. Rastogi, T.-C. Cheng and J.J. DeFrank. "Effect of temperature and pH on the secondary structure of organophosphorus hydrolase (OPH) by circular dichroism". 228th ACS National Meeting (Philadelphia, PA, August 22-26).

2003

7. Leblanc, R.M., C.A. Constantine, V. Rastogi, T.-C. Chen, J.J. DeFrank. "Organophosphorus hydrolase-based detection of organophosphorus agents". Joint Service Scientific Conference on Chemical and Biological Defense Research (Towson, MD, November 17-20).

8. Zheng, J., X. Cao, S.V. Mello and R.M. Leblanc. "ATR-FTIR study of organophosphorus hydrolase secondary structure of Langmuir-Blodgett films". 226th ACS National Meeting (New York, NY, September 7-11).

9. Constantine, C., P. Kele, J. Orbulescu, S.V. Mello and R.M. Leblanc. "Covalent immobilization of organophosphorous hydrolase for detection of organophosphorous compounds". Florida ACS Annual Meeting and Exposition 2003 (Clarion Hotel and Conference Center at Orlando International Airport, FL, May 8-10).

10. Zheng, J., X.Cao, S. V. Mello and R.M. Leblanc. "ATR-FTIR study of organophosphoprous hydrolase secondary structure of Langmuir-Blodgett films". Florida ACS Annual Meeting and Exposition 2003 (Clarion Hotel and Conference Center at Orlando International Airport, FL, May 8-10).

INVITED SPEAKER

2006

11. Leblanc, R.M. "Organized nano-films for bio-sensing". 19th “Entretiens Jacques Cartier” Nanobiotechnology for analysis and energy conversion (Grenoble, France, December 4-5).

12. Leblanc, R.M. "Organophosphorus-based assays for detection of organophosphorus compounds". 2006 Scientific Conference on Chemical and Biological Defense Research (Hunt Valley, MD, November 13-15).

13. Leblanc, R.M. and C. Wang. "Fluorescent bioassays based on organophosphorus hydrolase (OPH) for the detection of paraoxon". 58th Southeastern Regional Meeting of the American Chemical Society (Sermacs2006) (Augusta, GA, November 1-4).

14. Leblanc, R.M. and C. Wang. "Peptide bioassays for the detection of copper and paraoxon". 58th Southeastern Regional Meeting of the American Chemical Society (Sermacs2006) (Augusta, GA, November 1-4).

15. Leblanc, R.M., Plenary Lecture. "Biomolecular recognition and imaging using Quantum Dots". 8th International Conference on Fundamental and Applied Aspects of Physical Chemistry (Belgrade, Serbia, September 26-29).

16. Leblanc, R.M. "Molecular recognition at Langmuir monolayers". Department of Chemistry, Faculty of Science, Tokyo Institute of Technology, Tokyo, Japan (June 12).

17. Leblanc, R.M., Key Note Speaker. "Quantum dots as immunoassay probes". Particles2006. Medical/Biochemical Diagnostic, Pharmaceutical, and Drug Delivery Applications of Particle Technology (Orlando, FL, May 13-16).

18. Leblanc, R.M., Plenary Lecture. "Surface chemistry and spectroscopy towards sensing". Florida Annual Meeting and Exposition (Fame2006) (Orlando, FL, May 11-13).

19. Leblanc, R.M., C.A. Constantine, X. Ji and J. Orbulescu. "Bioassay development using organophosphorus hydrolase for detection of paraoxon". 209th Electrochemical Society Symposium (Denver, CO, May 7-11).

20. Leblanc, R.M. "Biomolecule recognition and imaging with peptides and quantum dots". Frontiers in Colloid, Surface and Supramolecular Chemistry in Biomedical Applications. 40th Western Regional Meeting of the American Chemical Society (Anaheim, CA, January 22-25).

2005
21. Leblanc, R.M., Plenary Lecture. "Surface manipulation and assemblies of quantum dots for chemo- and biosensing". Seven Annual Conference of the Yugoslav Materials Research Society (YUCOMAT 2005, Herceg Novi, Serbia and Montenegro, September 12-16).

22. Leblanc, R.M., Invited Speaker. "Quantum Dots: Self-assembly and Layer-by-Layer deposition towards sensor development". Workshop on Multifunctional Materials (DoD, Air Force) (Keystone, Colorado, August 21-26).

23. Leblanc, R.M., Key Note Speaker. "Surface manipulation and assemblies of quantum dots for chemo- and biosensing". International Conference on Research Trends in Science and technology (Beirut and Byblos, Lebanon, March 7-9).

2004
24. Leblanc, R.M., C.A. Constantine, J. Zheng, L. Zhao, V. Rastogi, T.-C. Cheng and J.J. DeFrank. "Spectroscopic detection of organophosphorus agents". 2004 Scientific Conference on Chemical and Biological Defense Research (Marriot Hunt Valley Inn, Hunt Valley, MD, November 15-17).

25. Leblanc, R.M., Key Note Speaker. "Langmuir, Langmuir-Bodgett and Layer-by-Layer films of biomacromolecules". The 57th Divisional Meeting on Colloid and Surface Chemistry. The Chemical Society of Japan with the 2004 Japan – Australia International Symposium (Tokyo University of Science, Yamaguchi, September 9-11).

26. Leblanc, R.M. "Circular Dichroism and Fourier Transform Infrared Spectroscopies of organophosphorus hydrolase". Department of Chemistry, Florida State University (Tallahassee, FL, August 27).

2003
27. Crespo, G., S.V. Mello, X. Cao and R.M. Leblanc. "Organophosphorous hydrolase immobilized via layer-by-layer technique for biosensor applications". 11th International Conference on Surface and Colloid Science (Iguassu, Brazil, September 15-19).

Number of Presentations: 27.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

N/A

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts): 0

Peer-Reviewed Conference Proceeding publications (other than abstracts):

N/A

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts): 0

(d) Manuscripts

N/A

Number of Manuscripts: 0.00

Number of Inventions:

Graduate Students

NAME	PERCENT_SUPPORTED
Xiaojun Ji	1.00
Jiayin Zheng	1.00
Liang Zhao	1.00
Changshan Wang	0.50
Kerim Gattas-Asfura	0.25
FTE Equivalent:	**3.75**
Total Number:	**5**

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Roger M. Leblanc	0.20	No
FTE Equivalent:	**0.20**	
Total Number:	**1**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED
Liat Corcia	0.20
FTE Equivalent:	**0.20**
Total Number:	**1**

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: .....

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:.....

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:.....

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale): .....

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering: .....

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense .....

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields: .....

Names of Personnel receiving masters degrees

NAME
Total Number:

Names of personnel receiving PHDs

NAME
Liang Zhao
Jiayin Zheng
Xiaojun Ji
Kerim Gattas-Asfura
Celeste A. Constantine
Xihui Cao
Total Number: 6

Names of other research staff

NAME	PERCENT_SUPPORTED	
Jhony Orbulescu	0.40	No
FTE Equivalent:	**0.40**	
Total Number:	**1**	

Sub Contractors (DD882)

Inventions (DD882)

RESEARCH ACCOMPLISHMENTS

This work was aimed at the study of the molecular interaction between organophosphorus (OP) compounds and the enzymes organophosphorus hydrolase (OPH) and organophosphorus acid anhydrolase (OPAA). The specific objectives were to: i) characterize the Langmuir monolayers of OPH and OPAA by studying their interfacial and spectroscopic properties; ii) investigate the molecular interactions between OPH and OPAA, respectively, with OP compounds using Polarization Modulation FTIR spectroscopy; iii) characterize the topography of the Langmuir-Blodgett (LB) films by scanning probe and environmental scanning electron microscopies; iv) characterize the basic features of an acetylcholinesterase (AChE) biosensor based on LB film technology; v) simulate the active site of AChE.

During the last three years we worked to achieve the above objectives. A summary of the results obtained related to each of the objectives is given below.

Objective i): **Characterization of the Langmuir monolayers of OPH and OPAA by studying their interfacial and spectroscopic properties**

The surface characterization of OPH Langmuir monolayers during paraoxon catalysis, revealed the formation of aggregates. Alterations to the micro-structural features of OPH monolayers were observed through surface pressure–area isotherm measurements, AFM, and *in situ* epifluorescence microscopy. Changes in pH, associated with the paraoxon hydrolysis, were indicated by fluorescence quenching of FITC tag on OPH macromolecule that can be monitored *in situ* by the epifluorescence microscopy. This work was published in *Colloids and Surfaces B: Biointerfaces, 2005, 40, 75-81.*

The pH and temperature effects on the secondary structure of OPH were studied in this work and overall analysis of the OPH CD spectra has provided information on the structural changes involved when the environment was altered. Native OPH has a significant percentage of α-helix regions and the most stable secondary structure of OPH solution can be obtained at pH 7.6, which is also the isoelectric point of the enzyme. When the pH changes, the α-helix is converted into β-strand conformation and the enzyme becomes insoluble. Physical instability such as aggregation and/or denaturation could account for the observed difference in the CD spectra. This result is in good accordance with the pH effect observed on the OPH surface pressure-area isotherm. The Langmuir-Blodgett (LB) film deposition technique preserves the enzyme secondary structure with respect to the solution. For the temperature effect, the optimum temperature observed for the OPH solution is 20 °C. Far-UV CD spectra of OPH solid samples displayed no significant change in band shape or position over the temperature range from 20 to 60 °C; however, some variations in intensity were observed. The thermal stability of the OPH LB and dry films is much improved compared to that of the OPH solution, which is interpreted as being due to a close molecular arrangement of the enzyme. This indicates that the secondary structure of OPH can be stabilized when it is immobilized as LB or dry films. These results were published in *J. Phys. Chem. B, 2004, 108, 17238 -17242.*

The molecular interaction between OPAA and DFP was studied in aqueous solution and at the air-water interface as well as the optimum conditions for stable Langmuir monolayer formation of OPAA. The characterization of OPAA aqueous solution and OPAA monolayer during DFP hydrolysis by spectroscopic methods revealed a change in OPAA secondary structure. The CD and Infrared Reflection Absorption Spectroscopy (IRRAS) spectra indicate that a definite loss of secondary structure was observed at 1.1×10^{-3} M of DFP. The quenching of fluorescence intensity can be used as an effective biosensor with a much lower detection limit of 10^{-6} M of DFP because fluorescence spectroscopy is a more sensitive system of detection relative to CD and IRRAS spectroscopies. This work was reported in *Biomacromolecules, 2005, 6, 1555 - 1560.*

Objective ii): Investigation of the molecular interactions between OPH and OPAA, respectively, with OP compounds using Polarization Modulation FTIR spectroscopy

The secondary structure of OPH was investigated at the air-water interface using Polarization Modulation-Infrared Reflection Absorption Spectroscopy (PM-IRRAS). The shape and position of the amide I and amide II bands were used to estimate the surface conformation and orientation of OPH. The PM-IRRAS results indicated that the enzyme did not unfold for the surface pressure used (0-30 mN/m). At low surface pressures the signal of amide I was very weak and the intensity was almost the same with amide II. Upon further compression, the PM-IRRAS signal and the ratio of the intensity of amide I and amide II both increase, implying an increased interfacial concentration of the enzyme. From the amide I/amide II ratio and the band position, it can also be deduced that the enzyme adopts an organization which gives a higher occupied surface at low surface pressure and rotates to a more vertical conformation at high surface pressure. The decompression of the OPH monolayer indicated that the behavior of the secondary structure at the air-water interface was reversible. PM-IRRAS was also used to investigate the pH effect of the subphase on the secondary structure of OPH. The secondary structure of OPH at the air-water interface was well defined when the pH of the subphase was near its isoelectric point (IP, pH 7.6). However, it adopted a different orientation when the subphase pH values were higher or lower than the IP with formation of random coil structure. The hydrolysis of organophosphorus compound paraoxon by OPH was also studied at the air-water interface by PM-IRRAS. The pH effect and the interaction with paraoxon both seemed to orientate the enzyme more in the plane of the interface and to produce random coil structure. This work was published in *Biomacromolecules, 2006, 7, 2806-2810.*

A similar approach was carried out for OPAA using PM-IRRAS and IRRAS. Similar features were observed in terms of secondary structure. The investigation included different incident angles. Some changes were observed upon presence of DFP in the subphase. The manuscript is *in preparation*.

Objective iii): Characterization of the topography of the Langmuir-Blodgett (LB) films by scanning probe and environmental scanning electron microscopies

Single OPH monolayer was physically adsorbed onto the surface of the silanized quartz slide as ultra-thin LB film. The detection was made using the FITC-labeled enzyme. Other factors included deposition surface pressure to find a proper pressure with maximum detection. The bioassay assembly developed by the LB deposition technique showed the advantages of quick response time, reproducible signal and multiple time detections. This work was reported in *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2004, 250, 349-356*.

Layer-by-Layer deposition

Following LB deposition a different technique was tried, namely Layer-by-Layer deposition of chitosan / thiophene polymer/OPH. The results were published in *J. Am. Chem Soc. 2003, 125(7), 1805-1809*. Another LbL system was worked out. For this purpose a polyelectrolyte architecture was fabricated that was composed of chitosan and organophosphorus hydrolase polycations along with thioglycolic acid-capped CdSe quantum dots (QDs) as the polyanion. This film was imaged by epifluorescence microscopy. UV-vis and emission spectroscopies were used to monitor the growth of the bilayer film due to the enhanced optical property of QDs. Photoluminescence of the functionalized QDs improved when sandwiched between the polycations layers. The presence of organophosphorus compounds was confirmed through photoluminescence spectroscopy. These results were published in *J. Phys. Chem. B, 2003, 107, 13762 - 13764.* The full study using this novel approach was published in *Langmuir, 2003, 19, 9863 -9867.*

OPH-QDs direct conjugation

Since the results using QDs were promising we tried to directly attach OPH on the surface of the QDs using electrostatic interactions. The OPH was coupled to (CdSe)ZnS core-shell QDs through electrostatic interaction between negatively charged QDs surfaces and the positively charged protein side chain and ending groups ($-NH_2$). Circular dichroism (CD) spectroscopy showed no significant change in the secondary structure of OPH after the bioconjugation, which indicates that the activity of OPH was preserved. Detectable secondary structure changes were observed by CD spectroscopy when the OPH/QDs bioconjugate was exposed to organophosphorus compounds such as paraoxon. Photoluminescence (PL) spectroscopic study showed that the PL intensity of the OPH/QDs bioconjugate was quenched in the presence of paraoxon. The overall quenching percentage as a function of paraoxon concentration matched very well with the Michaelis-Menten equation. This result indicated that the quenching of PL intensity was caused by the conformational change in the enzyme, which is confirmed by CD measurements. The detection limit of paraoxon concentration using OPH/QDs bioconjugate was about 10^{-8} M. Although increasing the OPH molar ratio in the bioconjugates will slightly increase the sensitivity of biosensor, no further increase of sensitivity was achieved when the molar ratio of OPH to QDs was greater than 20 because the surface of QDs was saturated by OPH. These results were published in *J. Phys. Chem. B, 2005, 109, 3793 -3799.*

QDs characterization

The surface chemistry study of the QDs was also investigated. For this purpose trioctylphosphine oxide (TOPO) capped CdSe and (CdSe)ZnS quantum dots (QDs) were prepared. The surface chemistry behavior of both QDs at the air–water interface was carefully examined by various physical measurements. Stable Langmuir films were formed for both QDs. The average limiting nanoparticle area derived from the π–A isotherms of the QDs could be used to estimate the average size of the QDs if the thickness of TOPO shell was counted. The epifluorescence image of the QDs Langmuir films revealed an aggregation in 2D during the early stage of the compression process. On the other hand, a diacetylene-peptide (PDA-Cys-Cys-Gly-OH) derivative, namely PDA-CCG, was used to modify the surface of QDs. This modification resulted in a robust and homogeneous Langmuir monolayer, which is photopolymerizable and contains active binding sites for the binding of biomolecules. This work was published in *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2006, 284-285, 35-42.*

Objective iv): Characterization of the basic features of an acetylcholinesterase (AChE) biosensor based on LB film technology

Regarding the objective using AChE biosensor based on LB deposition, we have decided to shift the focus towards an assay based on OPH. The reasons for this change are related to the low stability of the LB film upon washing with buffer solution between measurements. Second, because AChE is inhibited by paraoxon the bioassays cannot be used for repeated measurements and by the inhibition of the enzyme no paraoxon will be decomposed. Taking these factors into account and knowing that the OPH hydrolyses paraoxon giving p-nitrophenol that can be monitored by UV-vis spectroscopy we have decided to chemically attach OPH onto a quartz slide and try to detect paraoxon. For this purpose, the substrate was cleaned and modified prior to chemical attachment. Each modification step was monitored by imaging ellipsometry as the thickness increased with each modification step (see Figure below). The chemically attached OPH was labeled with a fluorescent dye (7-isothiocyanato-4-methylcoumarin) for the detection of paraoxon in aqueous solution, ranging from 10^{-9} to 10^{-5} M. UV-vis spectra were also acquired for the determination of the hydrolysis product of paraoxon, namely p-nitrophenol. This work was published in *Analytical Chemistry, 2006, 78, 7016-7021.*

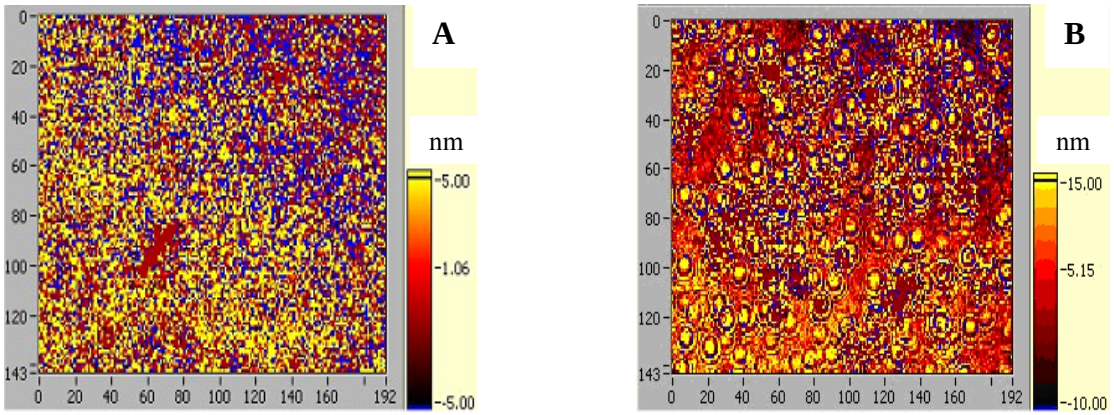
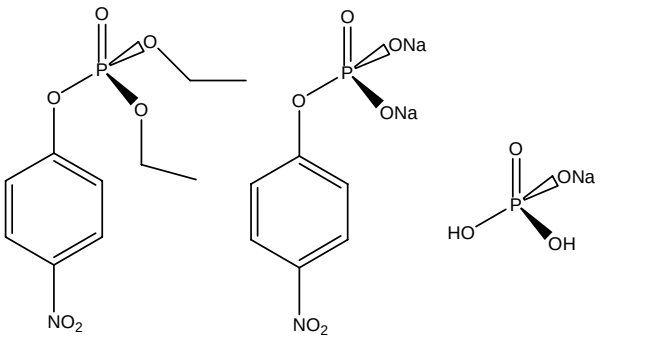


Figure. Ellipsometric characterization of silanized quartz slide (A) and covalently bound OPH.

Objective v): Simulation of the active site of AChE

Even though it was not mentioned at the time of the proposals' submission we decided to investigate the possibility of forming self-assembled structures from peptidolipids that could mimic the binding site of AChE.

We tried to use a two dimensional approach to mimic the active site of AChE using peptidolipids. For the peptide moiety we used amino acids involved in the binding site of AChE. Surface pressure-area and surface potential-area isotherms of the peptidolipid were studied on a pure water subphase. Paraoxon and two other small molecules, namely, sodium dihydrogen phosphate and 4-nitrophenyl phosphate disodium, which have a moiety of the structure of paraoxon, were solubilized in the subphase, but only paraoxon causes changes in the π-*A* isotherm of the peptidolipid. This indicated that there was a specific interaction between peptidolipid C_{18}-Phe-Trp-Ser-His-Glu (peptidolipid A) and paraoxon. Phe and Trp were responsible for the interaction because a second peptidolipid C_{18}--Gly-His-Ser-Glu-Glu (peptidolipid B), which has no aromatic amino acid, does not interact with paraoxon.



paraoxon **4-nitrophenyl phosphate disodium** **sodium dihydrogen phosphate**

Scheme 1. Chemical structures of paraoxon and two other analytes investigated for molecular recognition.

The compression-decompression cycles and long-term stability of the relevant peptidolipid Langmuir monolayer showed that the peptidolipid monolayer was stable. UV-vis spectra of the peptidolipid monolayer in the absence and presence of paraoxon were recorded. Although there was no change in the fingerprint of the spectrum in the presence of paraoxon, this result does not mean that there was no interaction. We used the tryptophan residue as an intrinsic probe of peptidolipid to observe an interaction between the peptidolipid and paraoxon. We observed a decrease of the intensity of fluorescence of Trp at 351 nm when the concentration of paraoxon was increased. This result supports our hypothesis on the interaction between the relevant peptidolipid and paraoxon. Epifluorescence micrographs of the peptidolipid mixed with a fluorescein derivative (molar ratio 100:1) in the absence and presence of paraoxon also support this hypothesis. This work was published in *Langmuir,* 2006, 22, 2200 -2204.

Note: As seen from the work performed most of the research was performed with OPH and paraoxon, only a small fraction being performed with OPPA and DFP. The reason is given by the spectroscopic methods employed, UV-vis and emission spectroscopies. The use of OPH to hydrolyze paraoxon has the great advantage of producing p-nitrophenol and diethyl phosphoric acid. This advantage was exploited in various ways: a) paraoxon UV-vis absorbance decrease upon hydrolysis followed with the increase in the absorbance of p-nitrophenol; b) increase of p-nitrophenol absorbance to cause fluorescence quenching using Förster fluorescence resonance energy transfer (FRET); c) formation of diethyl phosphoric acid causes a pH change of the enzyme

microenvironment. When QDs were used using different assay designs the increase in acidity causes quenching of the emission of QDs.
In the case of OPAA since only a fluoride ion is formed it was difficult to attain high sensitivity of detection using the ion selective electrode for example. One article was published on the surface chemistry, spectroscopy and AFM microscopy of OPAA Langmuir and LB films (*Biomacromolecules, 2003, 22, 2200-2204*).

SUMMARY

The US Army grant yielded in the complete solution and interfacial characterization of the OPH and OPAA enzymes and their interaction with paraoxon and DFP, respectively. It was shown that the LB film retains the secondary structure of the enzymes. A change in the surface topography was shown to occur when the LB films were exposed to analyte solutions.
The main findings of the research are related to the alternative deposition techniques that provide better stability, reproducibility and sensitivity as follows:

a) Layer-by-Layer deposition using QDs with a 10^{-9} M detection limit for paraoxon using OPH.

b) OPH-QDs direct conjugation with a 10^{-8} M detection limit for paraoxon.

c) Chemical attachment of OPH and labeling with a coumarin derivative with a 10^{-9} M detection limit for paraoxon.

d) Intrinsic fluorescence quenching of OPAA (tryptophan fluorescence) by DFP with a detection limit of 10^{-6} M.

These findings enable us to continue the research towards suitable bioassays using the techniques that yielded excellent sensitivity.